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SHORT COMMUNICATIONS

A New Field Method for Measuring Forest Litter Respiration Rate

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The forest litter is an important component of forest ecosystem: it regulates air, water, and heat regimes of the soil, contains a large stock of nutrients, and serves as a geochemical barrier to the input of pollutants from the atmosphere to underlying soil layers. In industrially polluted areas it can serve as an indicator of severity of disturbances in decomposition processes (Vorobeichik, 1995). The litter markedly contributes to the total CO_2 efflux from the soil (19–42%; Wang et al., 2009) and can be justly regarded as one of the main components of the corresponding soil system with specific mechanisms of functioning. To reliably model the carbon cycle on global and regional scales, it is necessary to accurately estimate CO_2 fluxes from different sources, including the litter. However, the CO_2 flux from this source is estimated vary rarely: the Scopus database contains more than 15000 papers on soil respiration (as of June 2015), but measurement of litter respiration is somehow addressed in only about 80 papers (data as of June 2015). Such a situation is not least because of insufficiently developed methodology of such measurements.

Methods for determining litter respiration in situ can be divided into direct (measurement of CO_2 efflux directly from the litter) and indirect (litter respiration is estimated by calculation as the difference between the total influx and that from horizons other than the litter) (Table 1). Direct measurements can be made either in isolated or in nonisolated litter.

In our opinion, an ideal method should satisfy several criteria: (1) unbiasedness of the results relative to the true value; (2) direct measurement of litter respiration; (3) the possibility of measurements in the field; (4) syntopy, or measurement of total CO_2 efflux and litter respiration in the same point; (5) synchronism, or simultaneous measurement of total CO_2 efflux and litter respiration; (6) rapidity; and (7) the possibility to simultaneously estimate several parameters of litter respiration (CO_2 efflux from the litter, its contribution to total efflux, and specific respiratory activity). Despite the diversity of approaches (Table 1), none of the existing methods satisfies the majority of these criteria.

We have developed and tested a new method for measuring litter respiration in the field (Smorkalov and Vorobeichik, 2012, 2016), which is as follows (Fig. 1). A steel collar 105 mm in diameter, with a sharp edge, is inserted in the soil to a depth of 3 cm, and the respirometer chamber is placed on it to measure total CO_2 efflux from the soil surface (Fig. 1a). At the next step, the collar is removed, and the cut-out fragment of the litter is transferred in a thin polyethylene bag, carefully but as quickly as possible. Then a clean polypropylene collar 105 mm in diameter and 50 mm high is placed in the bag so as to enclose the entire sample, and the litter is immediately put in its place, with the bag remaining open (Fig. 1b). After a certain period of time (determined empirically), the respirometer chamber is placed onto the polypropylene collar, tightly pressed against it to exclude air inflow from the outside, and then the rate of CO_2 from the litter is measured (Fig. 1c).

The key issue in implementing our method concerns the choice of appropriate time interval between measurements of total efflux and litter respiration. To obtain unbiased estimates, it is necessary to find a compromise between the waiting time for the litter to achieve stabilization of its respiration after unavoidable damage during transfer to the bag and the disturbing influence of the daily dynamics of CO_2 efflux, which is dependent mainly on fluctuations of soil temperature. The purpose of this study was to determine the minimum time interval necessary for stabilization of litter respiration in order to ensure adequate accuracy in measuring CO_2 efflux from the litter.

Studies were performed in August 2013 in three plots sharply differing from each other in litter stock (Table 2). The rate of CO_2 efflux was determined by the closed dynamic chamber method (Luo and Zhou, 2006) using a LI-8100A automated soil flux system

Table 1. Characteristics of different methods for measuring forest litter respiration

Description of the method (comments, major drawbacks)	1	2	3	4	5	6	References
Direct methods							
Comparing the isotopic composition of CO_2 emitted from normal and ^{13}C-depleted litter. *The litter is not isolated*. (The ^{13}C-depleted litter is prepared in the laboratory; the method is highly labor-intensive.)	+	–	±	–	+	–	Ngao et al., 2005; Joos et al., 2010
Measuring respiration of the litter *isolated* from other CO_2 sources. An acrylic vessel is dug in the soil and filled with ignited sand. The litter sample is placed on the sand and the respirometer chamber is put down on it. (Microclimatic conditions for the litter are altered.).	+	–	–	+	+	–	Ataka et al., 2014a
Measuring respiration of samples from different depths of the litter and individual leaves. *The litter is isolated.* Vertical variation in litter respiration is evaluated. (The litter is not in its normal state.)	–	–	–	–	–	+	Kominami et al., 2011; Ataka et al., 2014б
Indirect methods							
Comparing total CO_2 efflux with that from the soil surface devoid of the litter. Litter respiration is determined as the difference between total soil respiration and efflux from mineral horizons. (Microclimatic conditions are altered when the soil is exposed for a long time without the litter, which leads to distortion of the results.)	+	–	+	–	+	–	DeForest et al., 2009; Zimmermann et al., 2009; Berryman et al., 2014; Luo et al., 2014; Wu et al., 2014; Xiao et al., 2014
Removal of the litter. Its respiration is determined as the difference between total soil respiration and efflux from mineral horizons. (An interval of 1 to 24 h is necessary between measuring total CO_2 and litter respiration. Otherwise, the results will be strongly distorted: the contribution of mineral will be overestimated in the absence of the screening effect of the litter on CO_2 efflux from the soil.) (Smorkalov, 2011; Luo and Zhou, 2006)	–	+	–	+	+	–	Tewary et al., 1982; Metcalfe et al., 2007; Atarashi-Andoh et al., 2012
Comparing CO_2 efflux in plots with normal litter layer, without it, and with doubled litter layer. Liter respiration is determined by comparing CO_2 efflux in all three variants. (Microclimatic conditions are altered when the soil is exposed for a long with without litter or the amount of litter is increased.)	+	–	±	–	+	–	Boone et al., 1998; Sulzman et al., 2005; Prévost-Bouré et al., 2010; Zhang et al., 2014
Measuring respiration of mineral soil horizons from the walls of a soil pit. Contribution of the litter is determined as the difference between total efflux and efflux from mineral horizons. (Conditions of efflux are altered when the pit is dug, the method is labor-intensive.)	–	–	–	–	+	–	Kadulin and Koptsik, 2013

Designations: (1–3) characteristics of methods: (1) synchronism and (2) syntopy with measurements of total CO_2 efflux, (3) rapidity; (4–6) test parameters: (4) CO_2 efflux from the litter, (5) contribution of the litter to total CO_2 efflux, (6) specific respiratory activity of the litter.

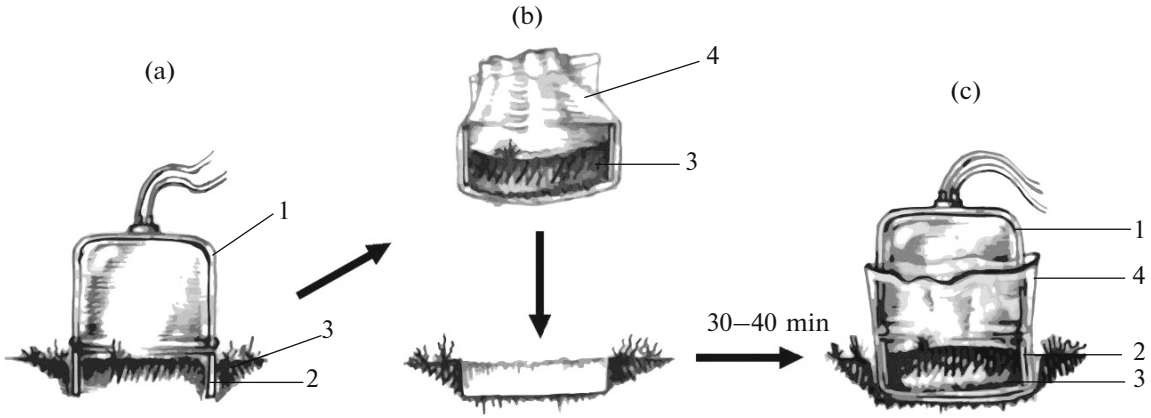

Fig. 1. Scheme of the method for measuring litter respiration: (a) measurement of total CO_2 efflux, (b) isolation of litter, (c) measurement of litter respiration; (1) respirometer chamber, (2) soil collar, (3) litter, (4) plastic bag.

(Li-Cor Bioscences, United States). Measurements in each plot were made in four to six randomly chosen points by the scheme described above. Measurements of CO_2 efflux from the litter were started immediately after bringing the bag with the litter sample back to the initial place and were made every 5 min for 2 h. The dynamics of efflux were recorded in an automatic mode: the instrument was programmed to carefully lift the chamber and put it down, which prevented additional damage to the litter. The time of one measurement was 1 min. The rate of efflux was estimated by a linear regression equation for the increase in CO_2 concentration in the system with regard to its volume, including the void space in the collar. A total of 350 measurements of litter respiration at 14 points were taken. After completing the procedure, the litter was brought to the laboratory, dried to air-dry state, and weighed to an accuracy of 0.01 g.

Additional experiments were performed to estimate possible CO_2 diffusion through polyethylene film that has already been in use. An empty bag with a polypropylene collar was placed on the soil surface, and single and serial measurements were performed in the automatic regime as described above. The results provided evidence only for slight fluctuations of CO_2 concentration in the system (±2 ppm).

To compare the litter respiration rate during each 5-min interval with the average respiration rates over

Table 2. Characteristics of test plots, $M \pm SE$

Parameter	Plot		
	S1	S2	P
Coordinates	56°48′24.29″ N, 59°21′43.96″ E	56°49′44.91″ N, 59°52′24.54″ E	56°41′14.41″ N, 60°55′16.51″ E
Biotope type	Nemoral–wood sorrel spruce–fir forest	Dead-cover spruce–fir forest	Herbaceous pine forest
Date of measurement	August 21, 2013	August 20, 2013	September 4, 2013
Number of points	6	4	4
Litter thickness (min–max), cm	1–2	7–11	1–4
Litter stock, kg/m^2	1.1 ± 0.1	7.4 ± 0.6	1.1 ± 0.3
Total efflux, mg CO_2/m^2/h	1219.7 ± 63.4	1964.2 ± 95.0	617.8 ± 47.5
Litter respiration*, mg CO_2/m^2/h	293.0 ± 31.7	1319.5 ± 228.1	82.4 ± 6.3
Specific respiratory activity of the litter*, mg CO_2/g/h	0.20 ± 0.02	0.13 ±0.02	0.07 ± 0.01

* According to the proposed method, the time interval between measurements of total CO_2 efflux and litter respiration was 30–40 min,

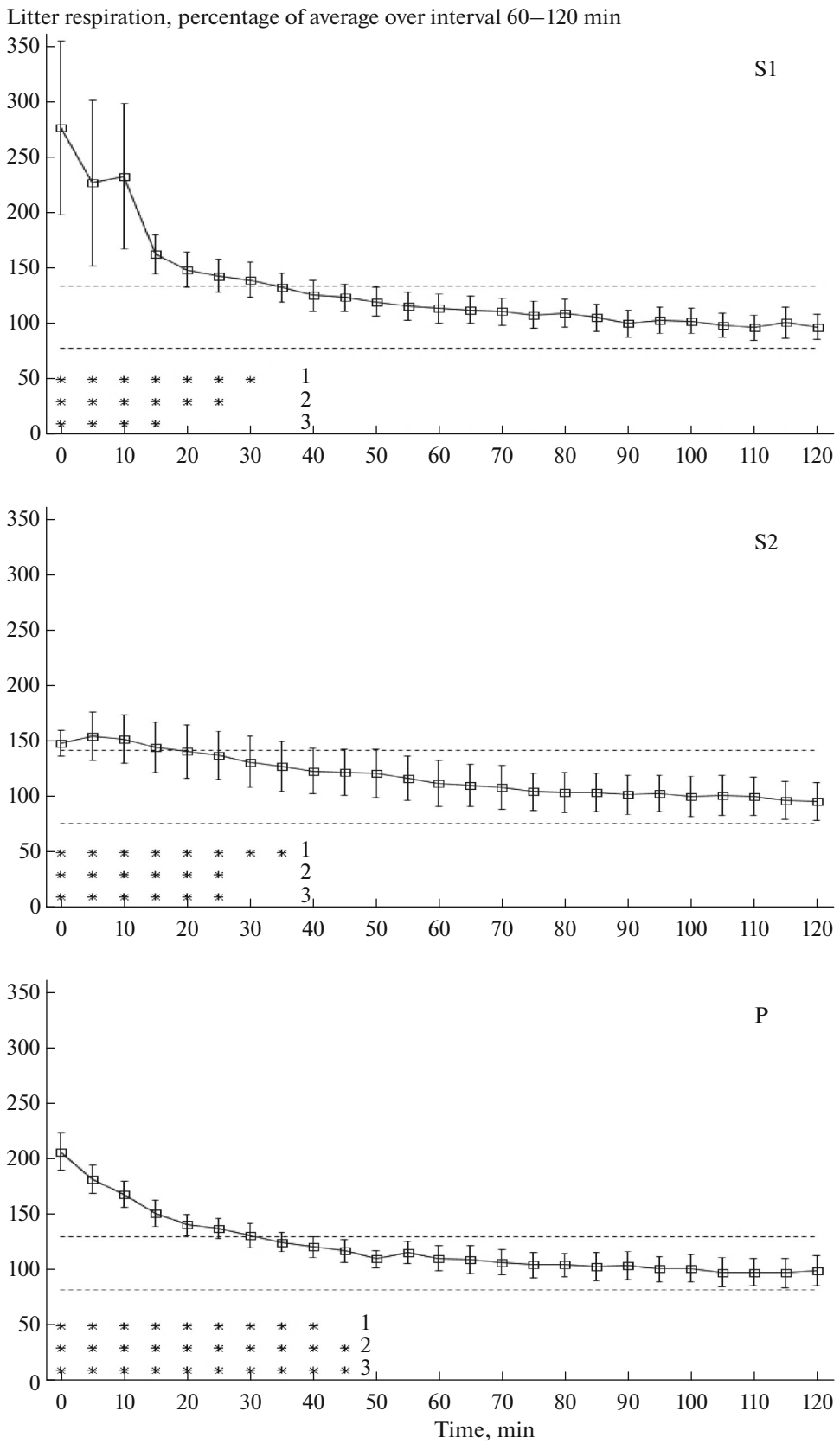

Fig. 2. Change in litter respiration rate after mechanical damage, $M \pm SE$. Dashed lines, average value over the interval 60–120 min ± 95% confidence interval. Asterisks indicate significant differences from average values over the intervals (1) 115–120 min, (2) 90–120 min, and (3) 60–120 min. S1, S2, and P designate test plots (see Table 2).

the past 5, 30, and 60 min of the 120-min measurement period, we used a simple (nonorthogonal) contrast ANOVA model in Statistica v. 8 program (StatSoft Inc., 2008).

After the litter is placed in the bag, its respiration rate rapidly decreases but reaches a plateau after 60 min and then remains almost unchanged (Fig. 2). Hence, the average respiration rate over the interval from 60 to 120 min can be taken as a true value and subsequently used as a reference.

Simple contrast ANOVA showed that differences from the reference value in the litter respiration rate lose statistical significance after 15–25 min in spruce forests and after 45 min in pine forest (Fig. 2). Data recorded after 30–40 min are overestimated by 26–32%, on average, relative to the reference value, but such an error in field measurements appears acceptable.

Thus, the requisite interval between measuring the total CO_2 efflux and litter respiration is 30–40 min. This is the minimum time that must elapse before it will be possible to obtain the results free of significant bias relative to the reference value (provided the number of replicates is sufficient). For higher accuracy, we recommend to use an interval of no less than 60 min. The proposed method has advantages over the others: it is sufficiently accurate, syntopic, prompt, and allows direct determination of both litter respiration and its contribution to the total CO_2 efflux and also th specific respiratory activity of the litter (respiratory rate per unit substrate mass). Conditions for the litter during measurements barely differ from those in the surrounding areas. To save time when using several ten of test points, measurement of total CO_2 efflux and litter respiration can be made consecutively: first, the total efflux is measured at 15–20 points, which takes 30–40 min, and then litter respiration is measured at th same points in the same order. The method is adapted to the Li-8100A system but can be easily modified and used with other instruments.

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